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Energetics of biomolecule adsorption on mesostructured cellular foam silica

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ABSTRACT

Heats of adsorption measured by flow microcalorimetry (FMC) were used to understand the energetics of biomolecule adsorption on mesostructured cellular foam (MCF) silica. Tryptophan (Trp), lysozyme (LYS), and bovine serum albumin (BSA) were used as probe molecules. The FMC results confirmed that attractive interactions (both electrostatic and van der Walls interactions) between the biomolecules and acid-washed MCF silica were the driving force for adsorption, even when the protein (BSA) and the surface were both negatively charged and repulsion interactions might be expected. Multiple exothermic events occurred, possibly because of multipoint interactions between biomolecules and MCF silica resulting from multiple binding sites on the biomolecule and a curved pore structure. Interestingly, the magnitude of the enthalpy of adsorption (ΔH_{Total}) increased with increasing biomolecule size at pH 5.2; the number of exothermic peaks corresponded to the number of binding regions on the biomolecule. In addition, standard Gibbs energies of adsorption ($\Delta G°$) and entropy of adsorption ($\Delta S°$) were calculated from batch adsorption isotherms and the measured enthalpy of adsorption. BSA adsorption energetics were significantly affected by changing the pH from 4 to 5.2. This effect was attributed to a dramatic conformational change of BSA as a function of pH. The energetics of adsorption provide invaluable insight into the mechanism biomolecule adsorption.

1. Introduction

Mesoporous silica materials are increasingly used to immobilize protein in protein chromatography [1–3], biocatalysis [4–7], and drug delivery [8–10] because these materials possess better physical properties (pore size, pore volume, and surface area) than conventional silica gel materials. Several types of mesoporous silica have been developed for these applications; excellent reviews [8,11–13] of their use and prospects are available. Since protein adsorption, desorption, and biocatalytic activity are influenced by the interactions between biomolecule and mesoporous silica surface, an understanding of the interactions is indispensable to the design of highly efficient adsorption systems.

A previous investigation of protein adsorption thermodynamics on mesoporous silica (SBA-15) demonstrated that adsorption is a complex phenomenon that can be affected by the nature of the biomolecule (adsorbate) as well as experimental conditions such as pH and temperature [14]. The pore structure and surface curvature of mesoporous silica can impact the biocatalytic activity of an immobilized enzyme [15]. Different mesoporous silica synthesis techniques yield different pore structures; these differences can be important to adsorption capacity and energetics. The impacts of synthesis conditions (acid concentration and aging time) and post-synthesis acid washing on the pore size and biomolecule adsorption capacity were recently reported [16]. In that study, reducing the window pore size of mesostructured cellular foam (MCF) silica did not reduce the adsorption capacity for a biomolecule until the pore size was comparable to the largest biomolecule dimension. The mobility of biomolecules in small pores indicated probable surface diffusion, in which biomolecules travel along the surface of the silica, rather than pore diffusion in which biomolecules anchor to a point of initial adsorption resulting in the blockage of narrow pores and reduced adsorption capacity [16]. In a previous study, nearly identical lysozyme adsorption capacities and enthalpies were observed for SBA-15 silica materials with pore sizes of 7.4 nm and 13 nm, but the lysozyme adsorption and enthalpy of adsorption were attenuated in 2.8 nm pores [17].

MCF silica has been used as an enzyme support for biocatalysis [4,18–21], in protein adsorption [22,23], and in chromatography [1], but the interactions between biomolecules and MCF silica during the immobilization process have not been studied extensively. Thermodynamic analyses of biomolecule adsorption have helped elucidate the intricate adsorption mechanisms in liquid chromatography, biomaterials, medicine, food, and nanotechnology

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[24,25]. Thermodynamic approaches have revealed unexpected phenomena: for example, both the adsorption of salivary proteins on hydroxyapatite and fluorapatite [26] and the adsorption of bovine serum albumin on an anion-exchange adsorbent are entropy-driven [27]. Thermodynamic properties (enthalpy, entropy, and free energy) can be estimated using either the Van't Hoff equation [28] or using calorimetric methods combined with a measured adsorption isotherm [29]. The calorimetric approach has proven to be the more reliable methodology at high biomolecule concentrations [30].

By measuring the heat released or absorbed, calorimetry allows calculation of free energy, enthalpy, and entropy changes during adsorption [28,31–33]. Calorimetric methods such as isothermal titration calorimetry (ITC) and flow microcalorimetry (FMC) rely on measurement of heat flows caused by interactions between biomolecules and adsorbent during protein adsorption. In ITC, the heat signal is measured as a biomolecule solution is added to a buffer solution with suspended adsorbent particles in a fed-batch cell. ITC has been used to provide mechanistic insight in separation/purification processes, drug development, and even in cell metabolism; an excellent review is available on these aspects [32]. On the other hand, flow microcalorimetry (FMC) measures the heat signals corresponding to adsorption and desorption in a small column, allowing an improved understanding of the driving forces and mechanisms for both adsorption and desorption [14,33,34]. In both ITC and FMC, the measured heat is the enthalpy change (ΔH) of adsorption. Unlike ITC, FMC is a fixed-bed process, permitting more dynamic heat signal measurements. FMC has been used to demonstrate the effect of crystallinity of the adsorbent (calcium hydroxyapatite) on protein adsorption: higher affinity of protein adsorption was observed as the adsorbent crystallinity increased [35]. Also, FMC experimentation has been used to reveal the complexity of an ion-exchange process; for example, an unexpected endothermic peak corresponding to a significant entropic change was observed [27]. In overloaded conditions (in the nonlinear adsorption at high adsorbate concentration) [27,34], FMC has resolved the underlying adsorption mechanisms despite a high degree of freedom in reactions due to highly non-ideal conditions.

Recent FMC studies investigated the effects of salt concentration [36] and surface modification [37] on protein adsorption on MCF silicas. One unexpected observation in that work was a consistently-observed second, time-delayed exotherm for the adsorption of lysozyme onto silica. As a relatively inflexible protein, lysozyme is not expected to undergo conformation changes during adsorption, leading the authors to postulate that a second adsorption site on lysozyme may be preferred at high surface concentrations of lysozyme. Based on the previously reported results [36,37], the mechanistic energetic studies were extended to include bovine serum albumin (BSA) and tryptophan. BSA is a large, flexible protein with multiple binding sites [38,39]; tryptophan is small and rigid. A better understanding of the underlying adsorption mechanisms can be gained using the thermogram patterns and heats of adsorption of these three biomolecular probes in conjunction with previous observations of biomolecule adsorption capacity on MCF silica [16].

2. Experimental

2.1. Materials

Lysozyme from chicken egg white (LYS, 90%), bovine serum albumin (BSA, 98%) and sodium acetate trihydrate (99%) were obtained from Sigma–Aldrich (St. Louis, MO). L-Tryptophan (Trp) was obtained from Fluka (99%). Hydrochloric acid and acetic acid were obtained from Pharmco-Aaper (Brookfield, CT). Sodium azide (Biotech research grade) was obtained from Fisher Scientific (Fair Lawn, NJ). MCF silicas (MCF-3A and MCF-3B) synthesized in a previous study [16] are used here; the silica had a cell diameter of 36 nm, window diameter of 15 nm, BET surface area of 770 $m^2\ g^{-1}$, and total pore volume of 2.4 $cm^3\ g^{-1}$. The difference in the textural properties (cell pore size, window pore size, surface area, and pore volume) of these two silicas was negligible for the purposes of this study.

2.2. Flow microcalorimetry (FMC)

Thermograms for biomolecule adsorption (Trp, LYS, and BSA) were collected at room temperature using flow microcalorimetry (Microscal FMC 3 Vi, Gilson Instruments, Westerville, OH, USA) following previously reported methods [27,28,40]. Briefly, the adsorbent was packed into a bed in the FMC and equilibrated overnight with flowing mobile phase. The adsorbate solution was injected into the sample loop upstream of the adsorbent bed. When the sample valve was opened, the protein solution flowed as a plug to the packed bed where thermistors recorded minute temperature changes. The volume of sample loop was 1.36 mL, the mobile phase flow rate was 1.9 mL h^{-1}, the bed volume was 0.17 mL, and the adsorbent sample mass was 27.6 ± 2.9 mg. The mobile phase was aqueous 0.1 M sodium acetate at pH 5.2 or pH 4 and adsorbate solutions were prepared by dissolving 8 mg mL^{-1} of each biomolecule in the mobile phase. Experimental data were fit to asymmetric Gaussian peaks using the PeakFIT software package (Systat Software Inc., San Jose, CA, USA); the integral heat of adsorption was calculated from the area of the deconvoluted peaks using a calibration factor measured separately for each mobile phase. The calibration factor was determined from the relationship between the peak areas corresponding to five heat pulses of known power and duration. In each calibration, peak area was linear with pulse energy (r^2 = 0.99). Each heat signal was normalized by the loading previously measured at the mobile phase concentration [16].

3. Results and discussion

The magnitude and chronology of thermal events during adsorption can help illuminate the adsorption mechanism [14,33,35]. Previous calorimetric studies of protein adsorption have revealed that exothermic events occurred when attractive forces between protein and surface dominated while endothermic events were related to lateral protein–protein interactions, protein restructuring (changes of conformation and orientation), and solvent release from the surface [34]. Likewise, a FMC study [14] showed that the adsorption of lysozyme (LYS) and bovine serum albumin (BSA) on large-pore (24 nm) mesoporous SBA-15 generates exotherms in most cases as a result of attractive interactions; differences in peak shape depend on the protein structure and adsorption mechanism.

In this study, thermograms for the adsorption of Trp (neutral biomolecule, pI 5.9), LYS (basic biomolecule, pI 10.8), and BSA (acidic biomolecule, pI 4.7) on MCF silica were measured using 0.1 M sodium acetate buffer at pH 5.2 and at pH 4. At pH 5.2 and 0.1 M ionic strength, LYS has a net positive charge of 1.5 [41], BSA has a negative charge of −1 [42] and the MCF silica has a surface charge density of −0.001 C m^2 [36,43]. At pH 4 and 0.1 M ionic strength, LYS has a net positive charge of 2 [41], BSA has a net positive charge of 3 [42], and the MCF silica carries a slight negative charge. Trp has a molecular weight of 204 g mol^{-1}; LYS and BSA are much larger with molecular weights of 14,700 and 66,000 g mol^{-1}, respectively. In addition to differences in isoelectric point and size, the biomolecules differ in flexibility. Trp can experience bond rotation, but does not have the bulk or complexity to undergo intramo-